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Synthesis and Bio-Evaluation of Some New Phenyl Pyrazoline Derivatives from Pera-Chloro Benzaldehyde Moiety

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ABSTRACT

A New series of 4-(4-Chloro phenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-phenyl- 4,5-dihydro-pyrazol-3-yl]phenyl} azetidin-2-one are synthesized by reacting 3-chloro-1-{4-[3-(Substituted phenyl)prop-2-enoyl]phenyl}-4-(4-Chloro phenyl) azetidin-2-one (0.01M) and phenyl hydrazine (0.01M) in presence of gl. Acetic acid. All these compounds were characterized by means of their IR, ^{1}H NMR, Spectroscopic data and microanalysis. All the synthesized products were evaluated for their antimicrobial activity. All the compounds were tested for their antibacterial and antifungal activities by broth dilution method.

Keywords: Chalcones, Phenyl Pyrazolines, azetidin-2-one, Antimicrobial activity.

1. INTRODUCTION

Considerable attention has been focused on Pyrazoline and substituted Pyrazoline due to their interesting biological activities. To synthesize Pyrazoline derivatives, we selected chalcones as starting material. Generally chalcones are 1, 3-diary l-2-propene-1-ones.

Some substituted Pyrazoline and their derivatives have been reported to possess some interesting biological activities such as anticancer[5], insecticidal[1], antibacterial[16], antifungal[14], antidepressant[3,10,11,17], anticonvulsant[2,12], anti-inflammatory[4], antibacterial[6] and antitumor[9] properties.

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Moreover, many selectively fluoro-substituted organic compounds show peculiar pharmacological and agrochemical properties[7,8,13,15].In the present study the reaction of 3-chloro-1-{4-[3-(Substituted phenyl) prop-2-enoyl] phenyl}-4-(4-Chloro phenyl) azetidin-2-one with phenyl hydrazine in presence of gl. Acetic acid to form Pyrazoline derivatives (**4a-j**). The structures of the various synthesized compounds were assigned on the basis of IR, ^{1}H-NMR spectral data and elemental analysis. These compounds were also screened for their antimicrobial activity.

2. EXPERIMENTAL

2.1 General

The IR spectra were recorded on IR affinity-1, DRS-8000A, Shimadzu, Ptc. Ltd., Japan spectrophotometer. The ^{1}H-NMR was recorded in DMSO on Bruker Advance II 400 MHz spectrometer using TMS as an internal standard. Melting points were determined in open capillary tubes and are uncorrected. The purity of the compounds was checked by TLC-using Silica gel-G (Merck). Column chromatography was performed on silica gel.

2.2 Preparation of 1-(4-{[(4-Chlorophenyl) methylene] amino}phenyl) ethanone (1)

A mixture of 4-Chloro benzaldehyde (0.01M), 1-(4-aminophenyl) ethanone (0.01M) and methanol (30ml) was heated for about 5 min. in a beaker (250 ml) to get a clear solution. The solution was kept overnight at room temperature to get the respective crude solid which was recrystallized from ethanol to obtain the pure crystals of 1-(4-{[(4-chloro phenyl) methylene]amino}phenyl) ethanone respect-tively. The yield of the product was 75% and the product melts at 120^{0}C. Found: C(69.88%) H(4.65%) N(5.41%) , Calcd. for $C_{15}H_{12}ClNO$: C(69.91%) H(4.69%) N(5.43%). IR, cm^{-1}: 3084(=C-H), 2922(-C-H), 1678(>C=O), 1628(>C=N-), 1595 (>C=C<), 1408(-CH_3, bend), 1301(-C-N<), 1240(-C-CO-C-), 738(-C-Cl). ^{1}H-NMR (DMSO, δ, ppm): 2.5785 (3H, s, $COCH_3$), 6.5144-7.7992 (8H, m, Ar-H), 8.803 (1H, s, -CH=N-).

2.3 Preparation of 1-(4-acetylphenyl)-3-chloro-4-(4-Chlorophenyl) azetidin-2-one (2)

In a 100ml Round bottom flask 1-(4-{[(4-Chloro phenyl) methylene] amino} phenyl) ethanone (0.01M) in 70ml benzene was taken. Chloro acetyl chloride (0.01M) was added at room temperature with constant stirring and triethylamine 1ml was added and the reaction mixture was refluxed for 7 hours. After the completion of reaction, solvent was removed by vacuum distillation. The solid was filtered, dried and recrystallized from toluene. The yield of the product was 60% and the product melts at 108^{0}C. Found: C(61.07%) H(3.88%) N(4.17%), Calcd. for $C_{17}H_{13}Cl_2NO_2$: C(61.10%) H(3.92%) N(4.19%). IR, cm^{-1}: 3041(=C-H), 2921(-C-H), 1712(>C=O), 1548(>C=C<), 1365(-CH_3, bend), 1292(-C-N<), 1197(-C-CO-C-),642(-C-Cl). ^{1}H-NMR (DMSO, δ, ppm): 2.5550 (3H, s, $COCH_3$), 4.8102 (1H, d, >CH-Ar), 5.4594 (1H, d, >CH-Cl), 7.3170-8.0618 (8H, m, Ar-H).

2.4 Preparation of 3-chloro-1-{4-[3-(Substituted phenyl) prop-2-enoyl] phenyl}-4-(4-Chlorophenyl) azetidin-2-one (3a-j)

To the solution of 1-(4-acetylphenyl)-3-chloro-4-(4-Chloro phenyl) azetidin-2-one (0.01M) in absolute ethanol (50 ml), substituted benzaldehyde (0.01M) and 2% NaOH were added and refluxed for 10 hours. After refluxing the reaction mixture was concentrated, cooled, filtered and neutralized with dil. HCl. The solid residue thus obtained was crystallized by absolute ethanol. IR(3d), cm^{-1}: 3043(=C-H), 1722(>C=O), 1624(>C=C<), 1451(-N=O), 1286(-C-N<), 1232 (-C-O-), 684(-C-Cl). ^{1}H-NMR (3g-DMSO, δ, ppm): 4.8757 (1H, d, >CH-Ar), 5.4224 (1H, d, >CH-Cl), 6.3621-8.5674 (12H, m, Ar-H), 7.9978 (2H, d, -CH=CH-), 9.9660 (1H, s, Ar-OH).

2.5 Preparation 4-(4-Chloro phenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-phenyl-4,5-dihydro-pyrazol-3-yl] phenyl} azetidin-2-one. (4a-j)

A mixture of 3-chloro-1-{4-[3-(Substituted phenyl) prop-2-enoyl] phenyl}-4-(4-Chloro phenyl) azetidin-2-one (0.01M) and phenyl hydrazine (0.01M) in gl. acetic acid (30ml) was refluxed in an oil bath at 110-1200C for 4 hours. The reaction mixture was poured over crushed ice; product was isolated and crystallized from ethanol.

IR (**4c**), cm^{-1}: 3367(-OH), 3051(=C-H), 2933(-C-H), 1731(>C=O), 1610 (>C=N), 1521(>C=C<), 1458 (-CH_2-), 1382(-CH_3-) 1294(-C-N<),1238 (-C-N), 1178 (-C-O-), 694(-C-Cl). ^{1}H-NMR (**4d**-DMSO, δ, ppm): 3.82 (2H, d, CH_2- of Pyrazol), 4.30 (1H, t, >CH-Ar of Pyrazol), 4.79 (1H, d, >CH-Ar of Azetidine), 5.21 (1H, d, >CH-Cl of Azetidine), 6.72-8.30 (17H, m, Ar-H, -NH-).

3. RESULTS AND DISCUSSION

3.1 Antimicrobial activity

The MICs of synthesized compounds were carried out by broth micro dilution method as described by Rattan (Rattan et al., 2000). It is one of the non automated in vitro bacterial susceptibility tests. This classic method yields a quantitative result for the amount of antimicrobial agents that is needed to inhibit growth of specific microorganisms.

The *in vitro* antimicrobial activity of test compounds were assessed against 24 hr cultures of several selected bacteria and fungi. The bacteria used were *E. coli*, *S.aureus, P. aeruginosa*, and *S. pyogenus*; the fungi used were *C. albicans, A. Niger, and A.clavatus.*

The antimicrobial activity was performed by broth dilution method in DMSO. Gentamycin, Ampicilin, Chloramphenicol, Ciprofloxacin, Norfloxacin, Nystatin and Greseofulvin were used as standard for the evaluation of antibacterial and antifungal activities respectively. The activity was reported by Minimal Inhibition Concentration. The results are summarized in Table-2.

Biological screening result of 4-(4-Chloro phenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-phenyl-4, 5-dihydro-pyrazol-3-yl] phenyl} azetidin-2-one based derivatives shows that compound **4c** have shown better activity against E. coli, while compound **4b**

& **4i** have shown better activity against S. Pyogenus, while rest of all compound possessed good activity against *S. aureus* in the range of 100-225 µg/ml and *P. aeruginosa* in the range 62.5-250 µg/ml. Compound **4c**, 4e and **4i** is found to be significant antifungal activity.

Table: 1: Physical constant of 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-phenyl-4, 5-dihydro-pyrazol-3-yl] phenyl} azetidin-2-one (4a-j)

Compd	R	M.F.	Yield %	M.P. °C	Elemental Analysis		
					% C Found (Calcd)	% N Found (Calcd)	% H Found (Calcd)
4a	-2-Cl	$C_{30}H_{22}Cl_3N_3O$	72	116	65.82 (65.89)	7.64 (7.68)	4.01 (4.05)
4b	-2-OH	$C_{30}H_{23}Cl_2N_3O_2$	74	118	68.15 (68.19)	7.91 (7.95)	4.32 (4.39)
4c	-3,4-$(OCH_3)_2$	$C_{32}H_{27}Cl_2N_3O_3$	71	107	67.10 (67.14)	7.31 (7.34)	4.71 (4.75)
4d	-3-NO_2	$C_{30}H_{22}Cl_2N_4O_3$	76	110	64.59 (64.64)	10.01(10.05)	3.94 (3.98)
4e	-4-Cl	$C_{30}H_{22}Cl_3N_3O$	69	128	65.82 (65.89)	7.64 (7.68)	4.01 (4.05)
4f	-4-$N(C_2H_5)_2$	$C_{34}H_{32}Cl_2N_4O$	72	113	69.95 (69.98)	9.56 (9.60)	5.50 (5.53)
4g	-4-OH	$C_{30}H_{23}Cl_2N_3O_2$	78	180	68.15 (68.19)	7.91 (7.95)	4.32 (4.39)
4h	-4-$N(CH_3)_2$	$C_{32}H_{28}Cl_2N_4O$	70	193	69.14 (69.19)	10.02 (10.09)	5.01 (5.08)
4i	CHO	$C_{30}H_{23}Cl_2N_3O$	79	154	70.30 (70.32)	8.16 (8.20)	4.48 (4.52)
4j	-2-OH-3-OCH_3	$C_{31}H_{25}Cl_2N_3O_3$	77	138	66.62 (66.67)	7.48 (7.52)	4.47 (4.51)

Table: 2 Antimicrobial activity of 4-(4-Chlorophenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-phenyl-4, 5-dihydro-pyrazol-3-yl] phenyl} azetidin-2-one

SR. NO.	COMP. NO.	R	ANTIBACTERIAL ACTIVITY MINIMAL INHIBITION CONCENTRATION				ANTIFUNGAL ACTIVITY MINIMAL INHIBITION CONCENTRATION		
			E.COLI	P.AERUGINOSA	S.AUREUS	S.PYOGENUS	C.ALBICANS	A.NIGER	A.CLAVATUS
			MTCC 443	MTCC 1688	MTCC 96	MTCC 442	MTCC 227	MTCC 282	MTCC 1323
1	4a	-2-Cl	175	200	175	250	800	700	700
2	4b	-2-OH	100	200	62.5	100	1000	1000	800
3	4c	-3-OCH_3, -4-OCH_3	250	200	250	250	1000	250	500
4	4d	-3-NO_2	200	250	200	250	1000	500	500
5	4e	-4-Cl	200	250	125	250	500	1000	1000
6	4f	-4-$N(C_2H_5)_2$	175	225	200	125	750	600	800
7	4g	-4-OH	200	200	225	200	1000	800	>1000
8	4h	-4-$N(CH_3)_2$	150	150	175	200	700	>1000	1000
9	4i	-H	125	200	100	100	250	1000	1000
10	4j	-3-OCH_3, -4-OH	200	250	125	250	800	800	800

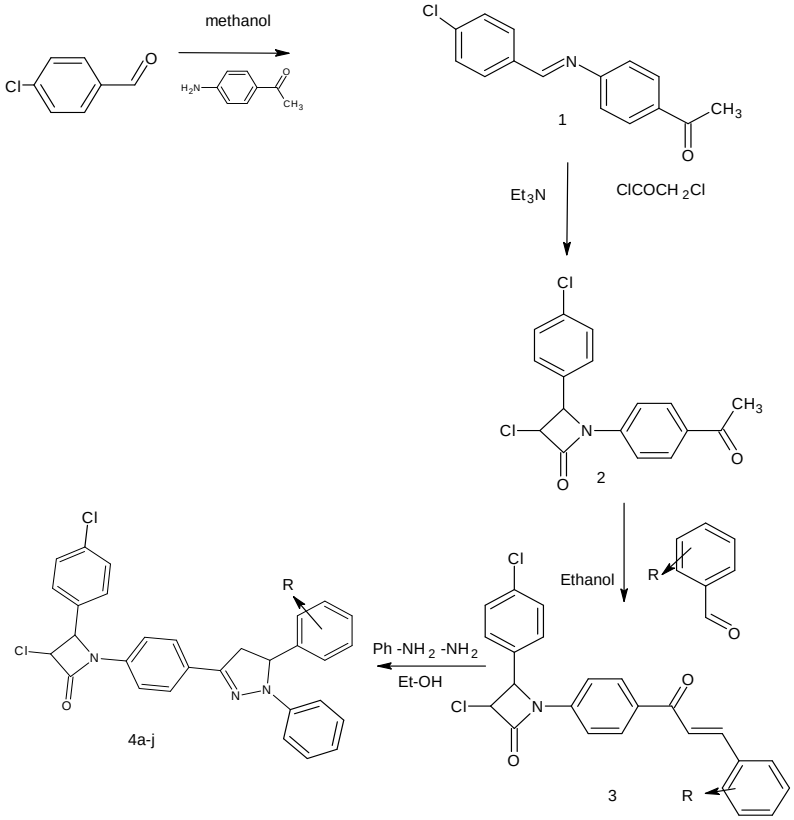
Table: 3 Antibacterial Activities: Minimal Inhibition Concentration (The Standard Drugs)

DRUG	E.COLI	P.AERUGINOSA	S.AUREUS	S.PYOGENUS
-	MTCC 443	MTCC 1688	MTCC 96	MTCC 442
(MICROGRAMME/ML)				
GENTAMYCIN	0.05	1	0.25	0.5
AMPICILLIN	100	--	250	100
CHLORAMPHENICOL	50	50	50	50
CIPROFLOXACIN	25	25	50	50
NORFLOXACIN	10	10	10	10

Table: 4 Antifungal Activity: Minimal Inhibition Concentration (The Standard Drugs)

DRUG	C.ALBICANS	A.NIGER	A.CLAVATUS
-	MTCC 227	MTCC 282	MTCC 1323
(MICROGRAMME/ML)			
NYSTATIN	100	100	100
GRESEOFULVIN	500	100	100

Reaction Scheme



4. CONCLUSION

The Main focus of this research work was to synthesize, characterize and evaluate antimicrobial activities of the newly synthesized Phenyl Pyrazoline derivatives, structures of synthesized compounds were confirmed and characterized with the help of analytical data's such as IR and ^{1}H-NMR. In summary, we have described the synthesis and antimicrobial activity of novel of 4-(4-Chloro phenyl)-3-chloro-1-{4-[5-(Substituted phenyl)-1-phenyl-4, 5-dihydro-pyrazol-3-yl] phenyl} azetidin-2-one MIC values revealed that amongst newly synthesized compound having Methoxy phenyl type linkage has shown good activity against the bacterial strains.

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